

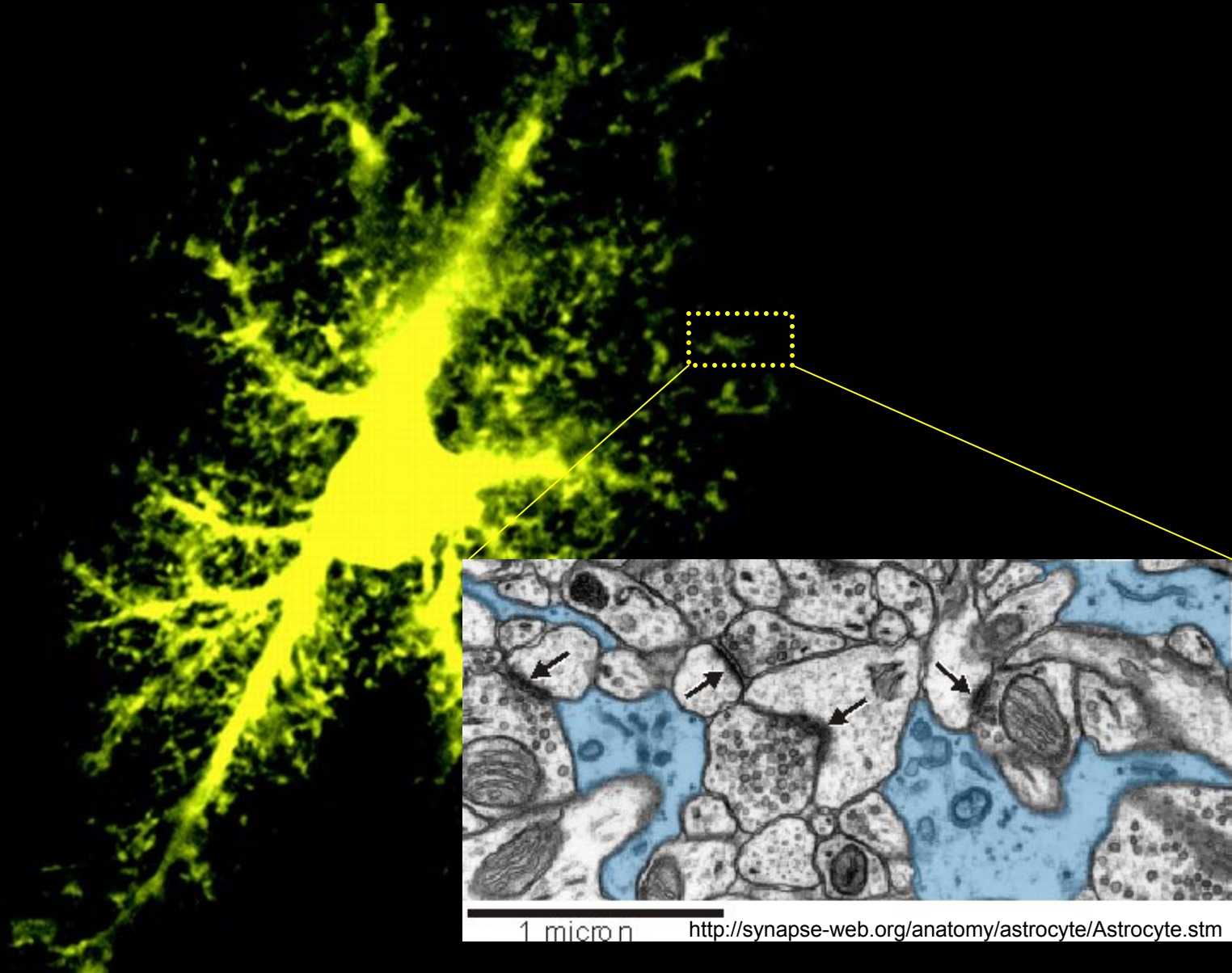
Glial Cell Induction and Suppression Of Neuronal Synapses

Çağla Eroğlu

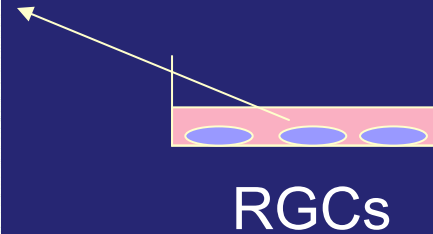
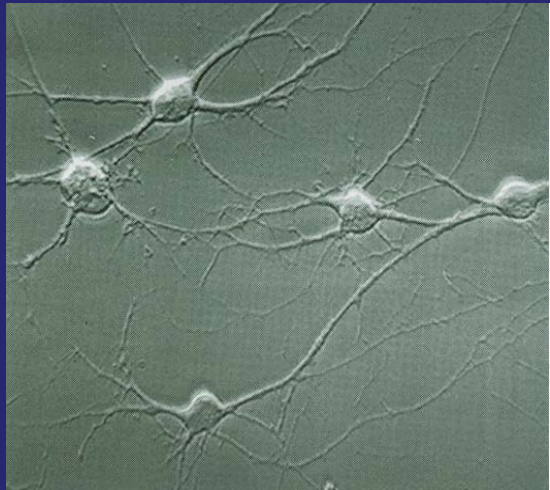
(Ben A. Barres)

Department of Neurobiology
Stanford University School of Medicine

What Do Astrocytes Do at Synapses?

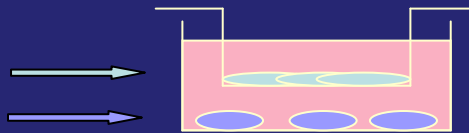


Retinal Ganglion Cells can be Cultured in the Absence and Presence of Glia

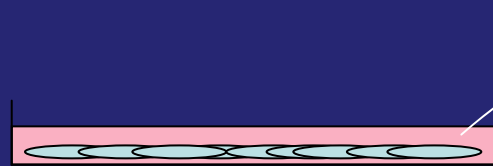


= “Control”

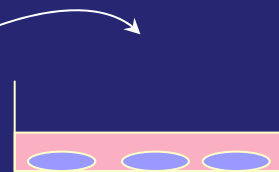
Astrocytes
RGCs



= “+ Astrocytes”



Medium conditioned
by astrocytes

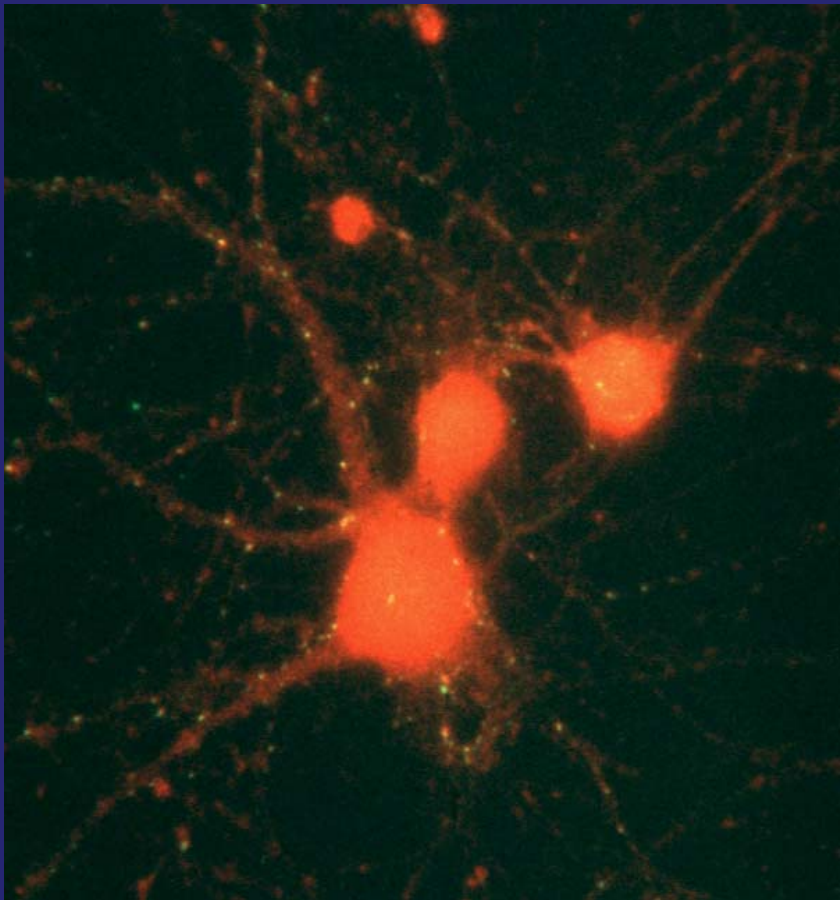


= “ACM”

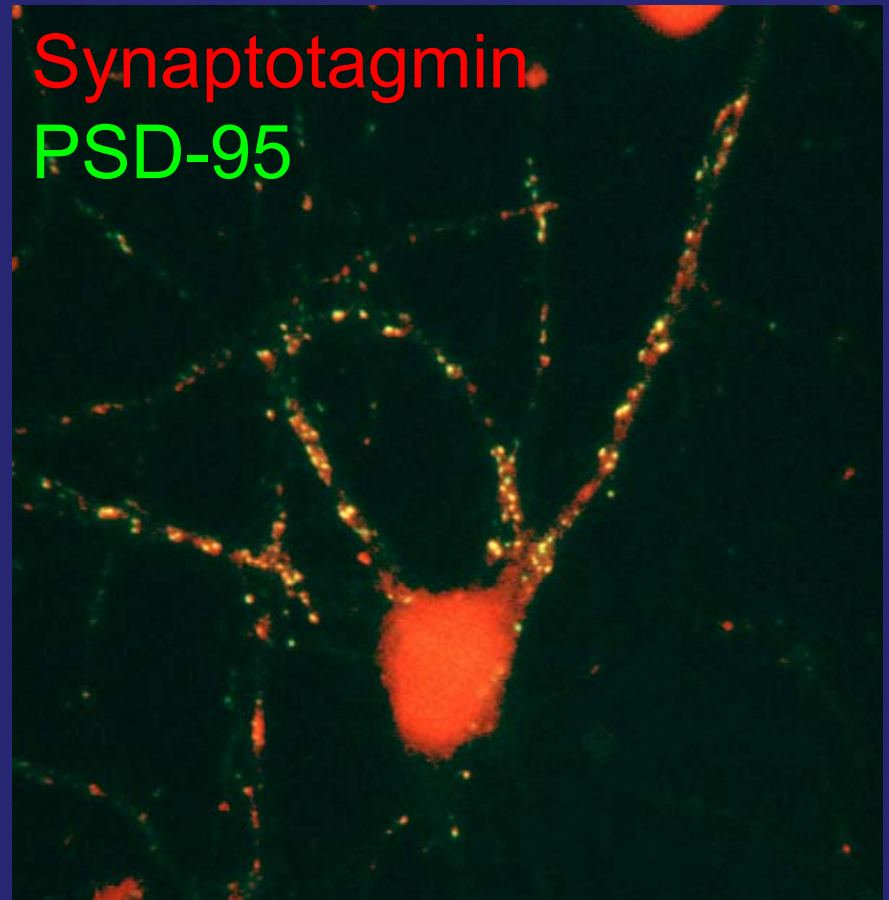


Astrocytes Induce Synapse Formation

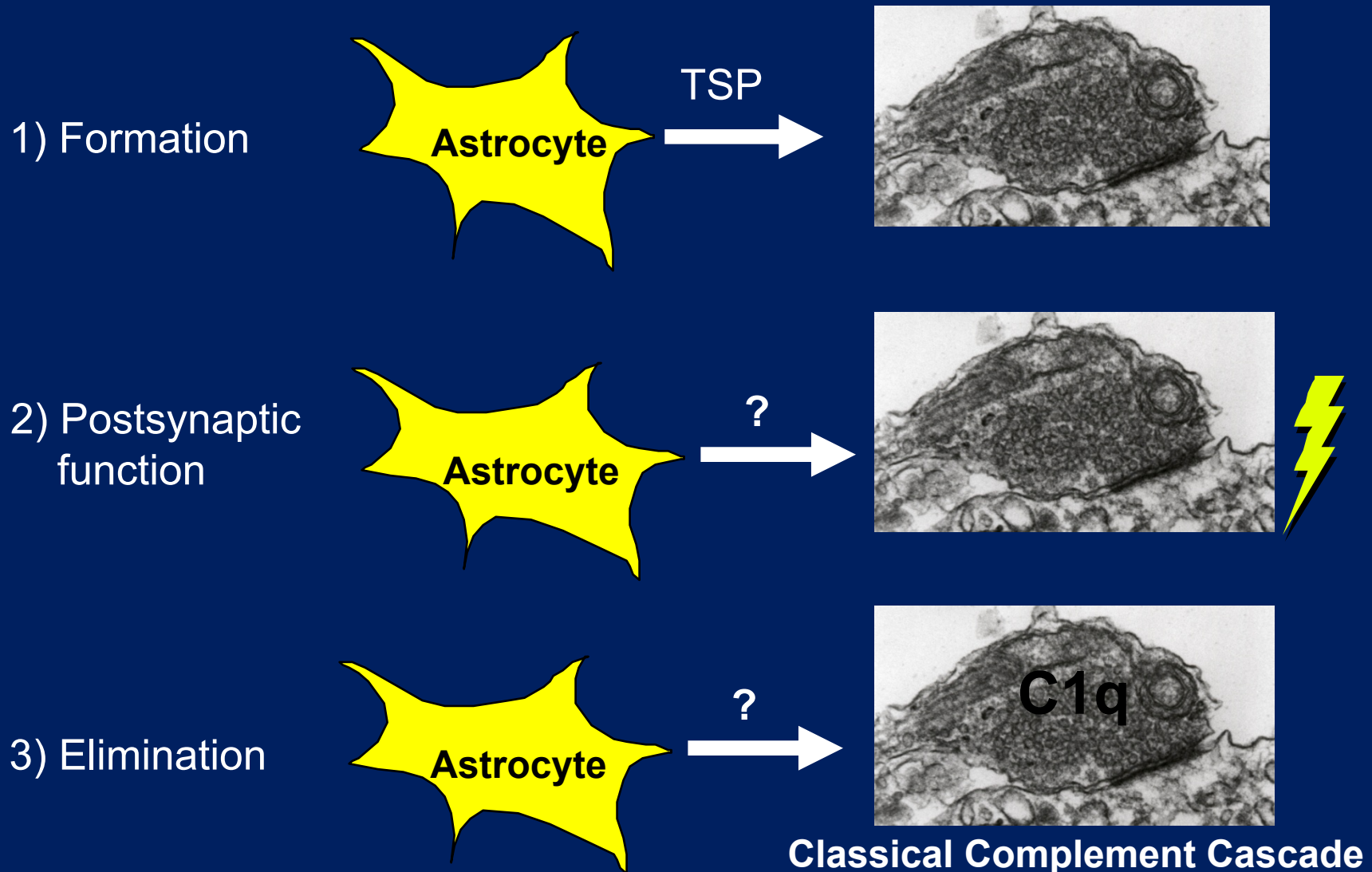
No Astrocytes



Astrocytes



Astrocytes Induce Formation of Fully Functional Synapses in Two Steps



How does Thrombospondin
Induce Synapse Formation?

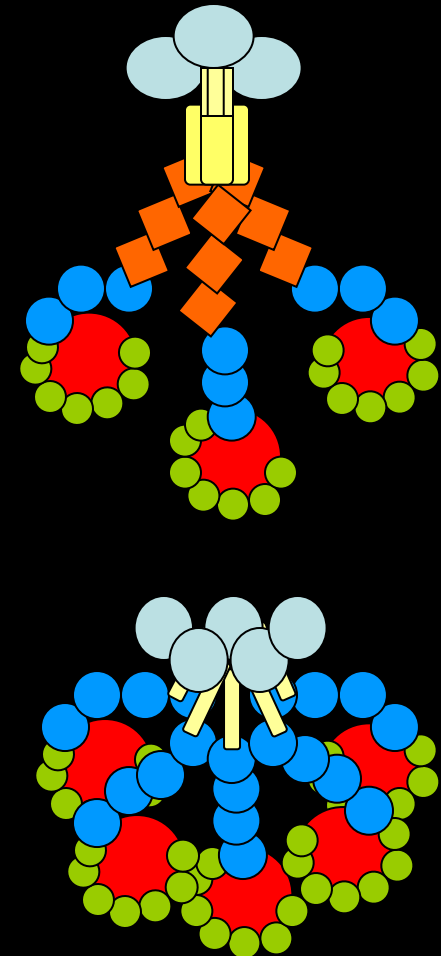
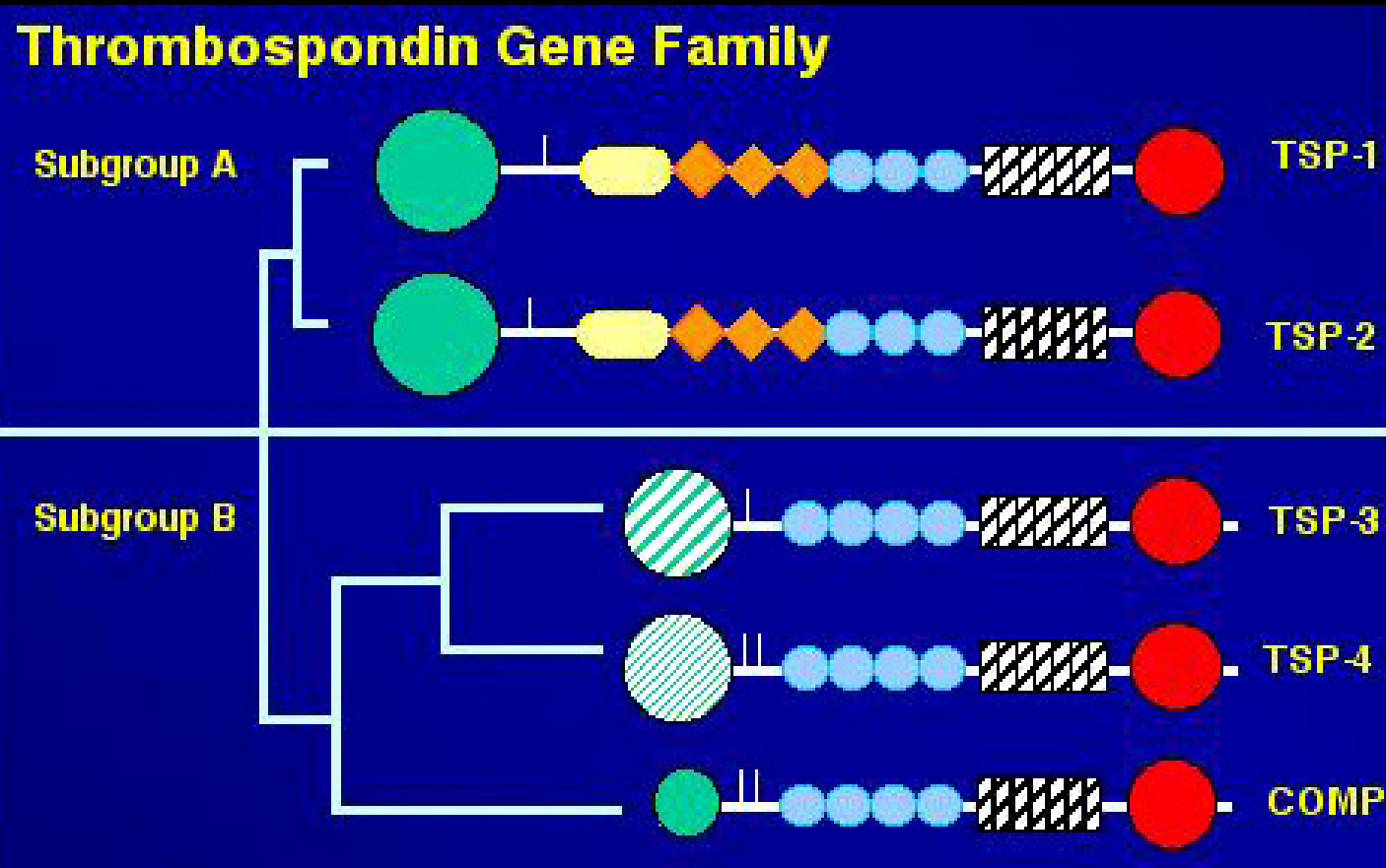
Çağla Eroğlu



**Starts Assistant Professorship this Spring
Department of Cell Biology, Duke University**

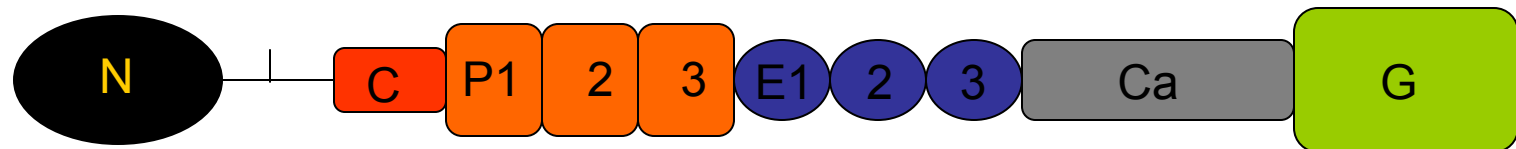
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What are Thrombospondins?

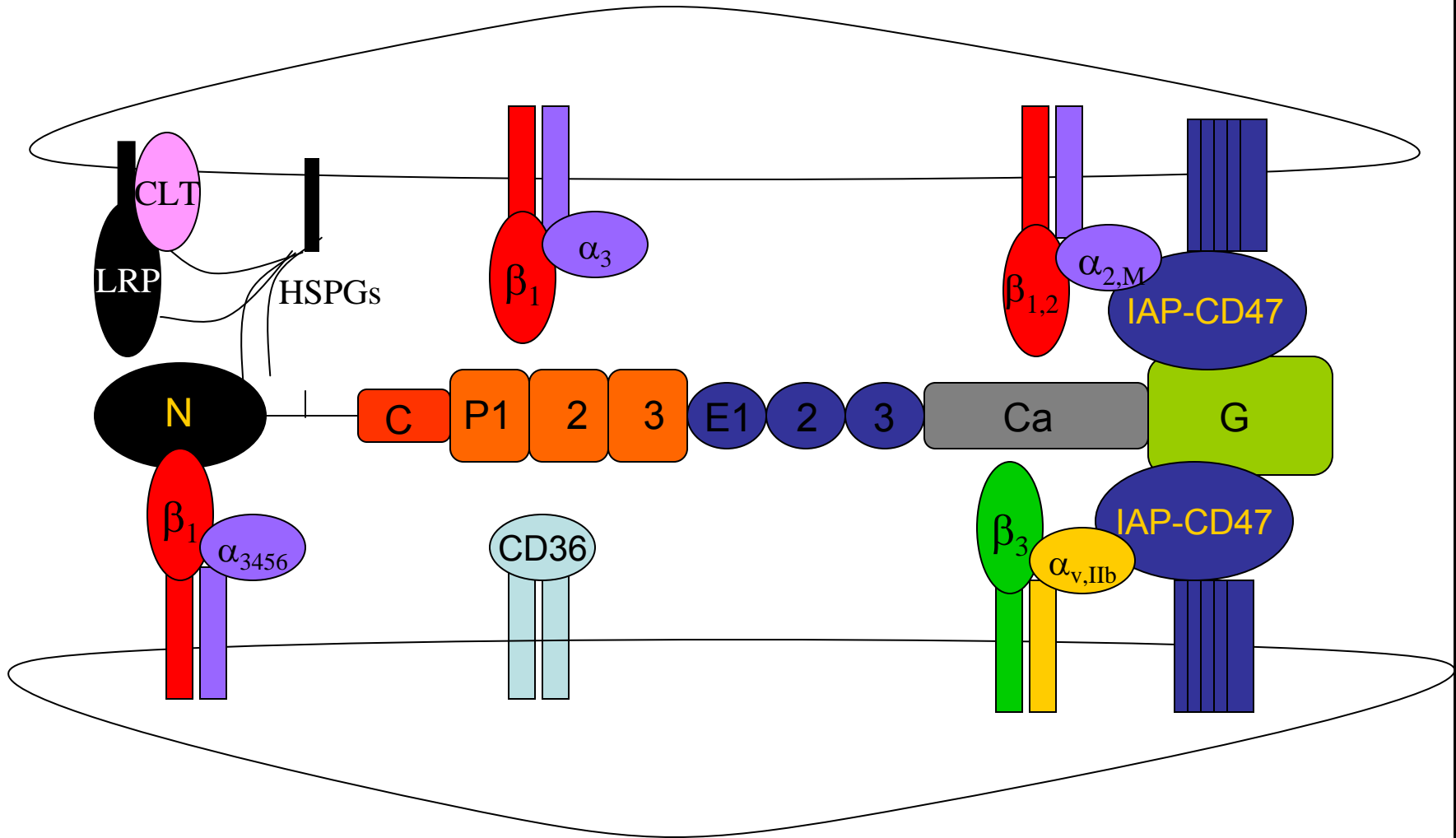


- TSP1 ,2 expressed by developing astrocytes; TSP4 at NMJ
- Regulate cell attachment, cytoskeleton, migration, and angiogenesis.
- TSP is the gene most upregulated in human vs monkey brain

TSP is Known to Interact with Many Cell Surface Receptors



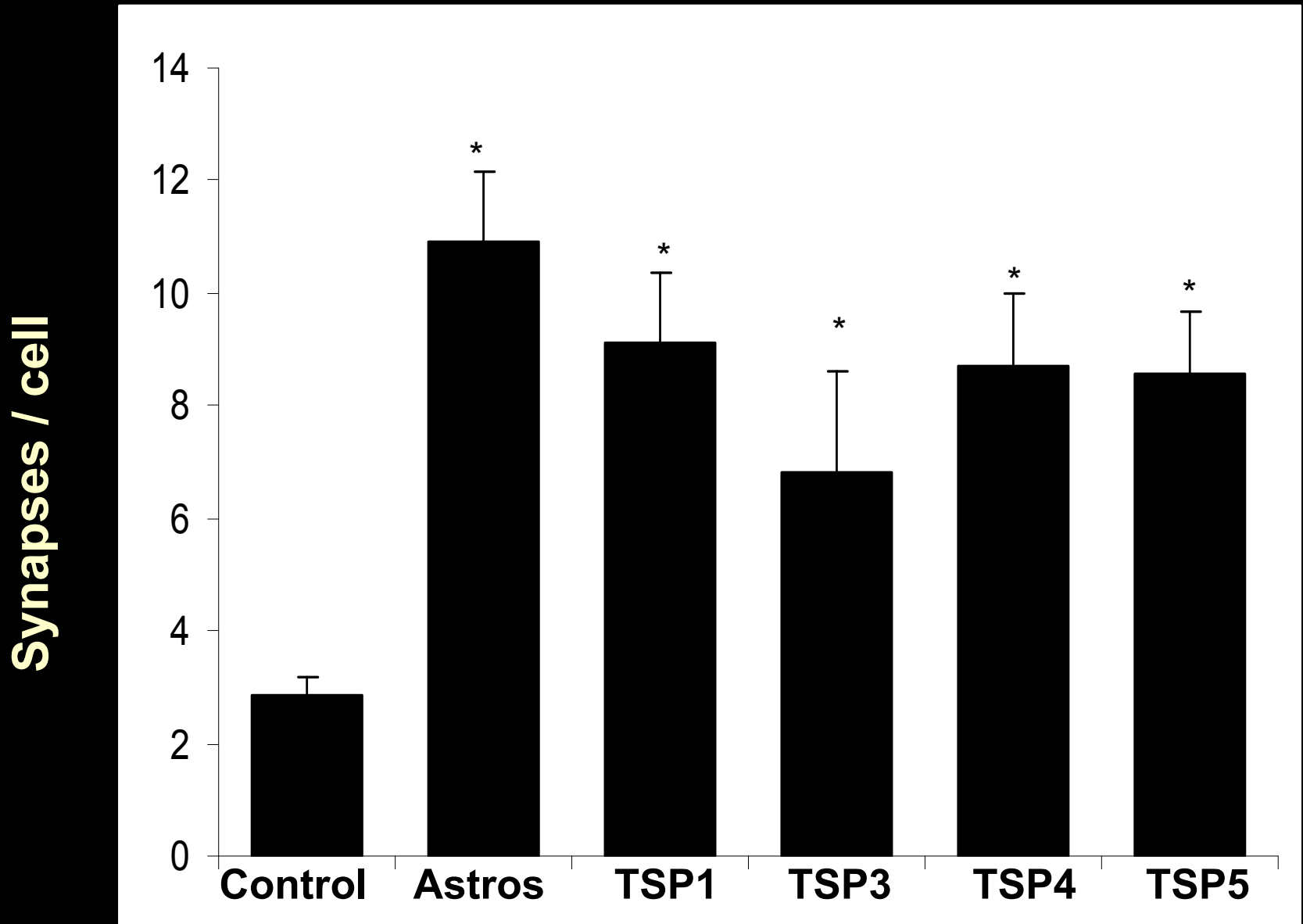
TSP is Known to Interact with Many Cell Surface Receptors



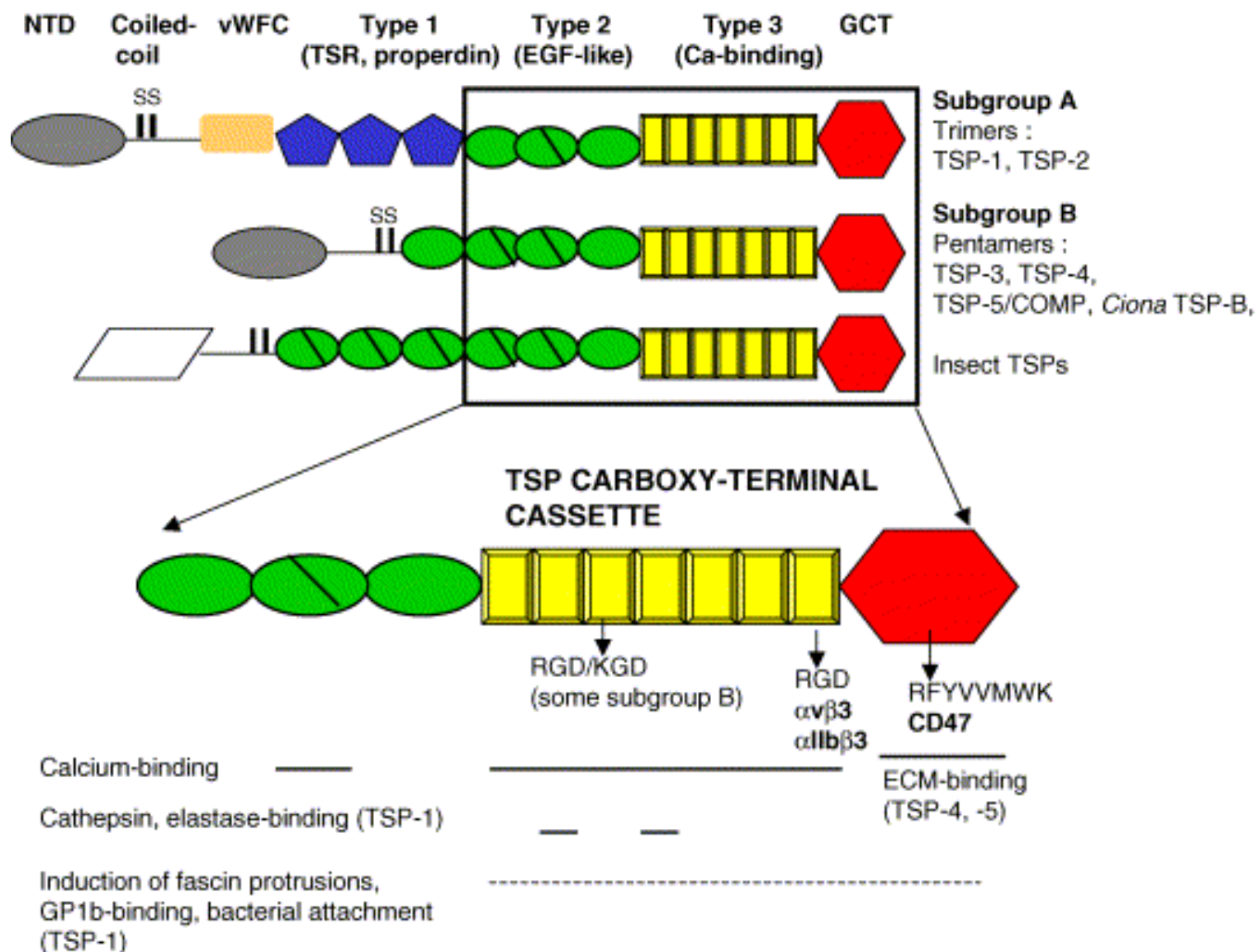
Strategy: Identify Neuronal TSP Receptor

- Are known TSP receptors expressed by RGCs and are they crucial for TSP induced synapse formation? **NO**
- Are all TSPs synaptogenic?
- Which domain of TSP is synaptogenic?

All Thrombospondin Isoforms are Synaptogenic



TSP ISOFORMS SHARE COMMON C-TERMINAL DOMAINS

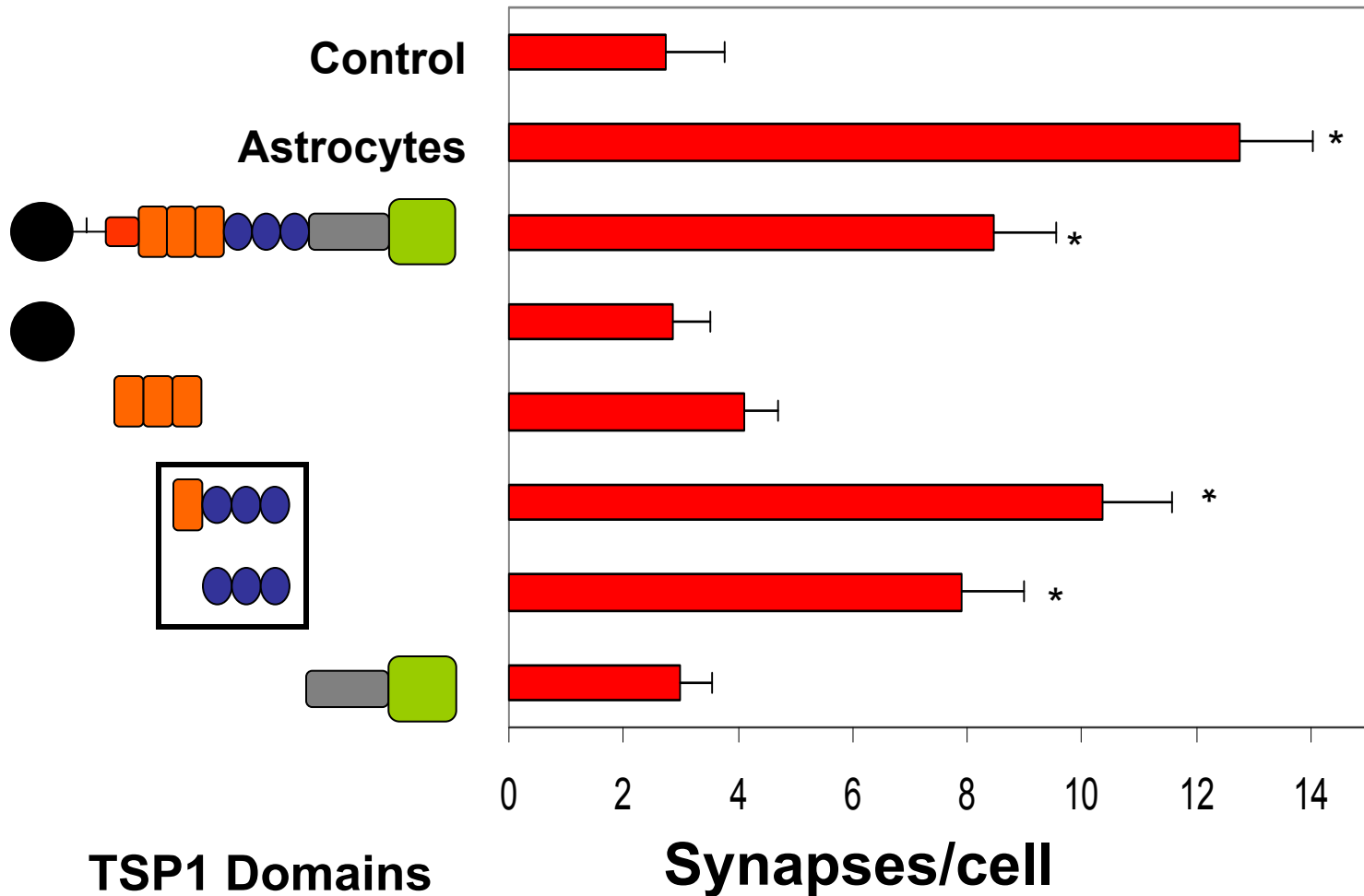


Deane F. Mosher

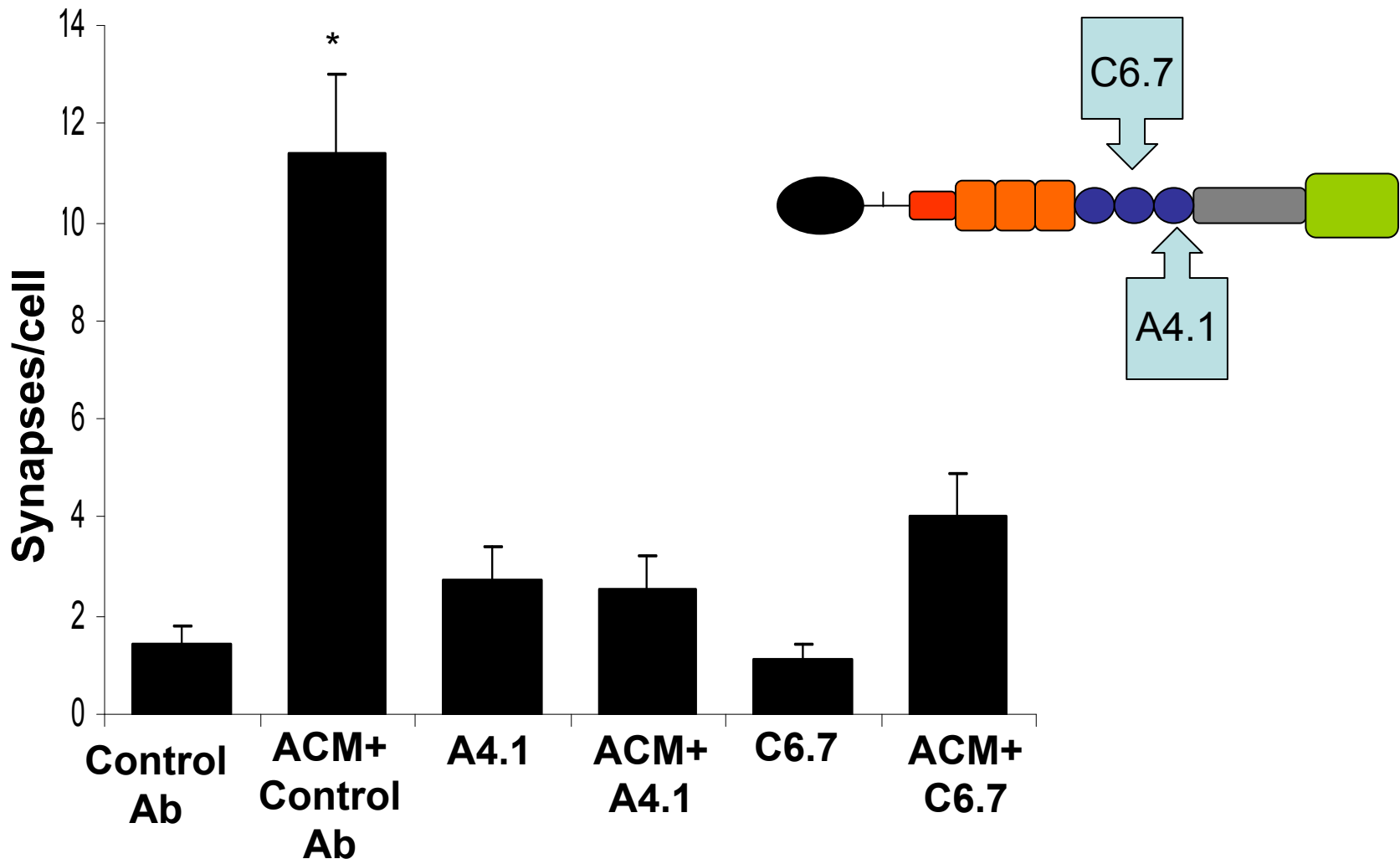


**Departments of Medicine and Biomolecular Chemistry
University of Wisconsin -- Madison**

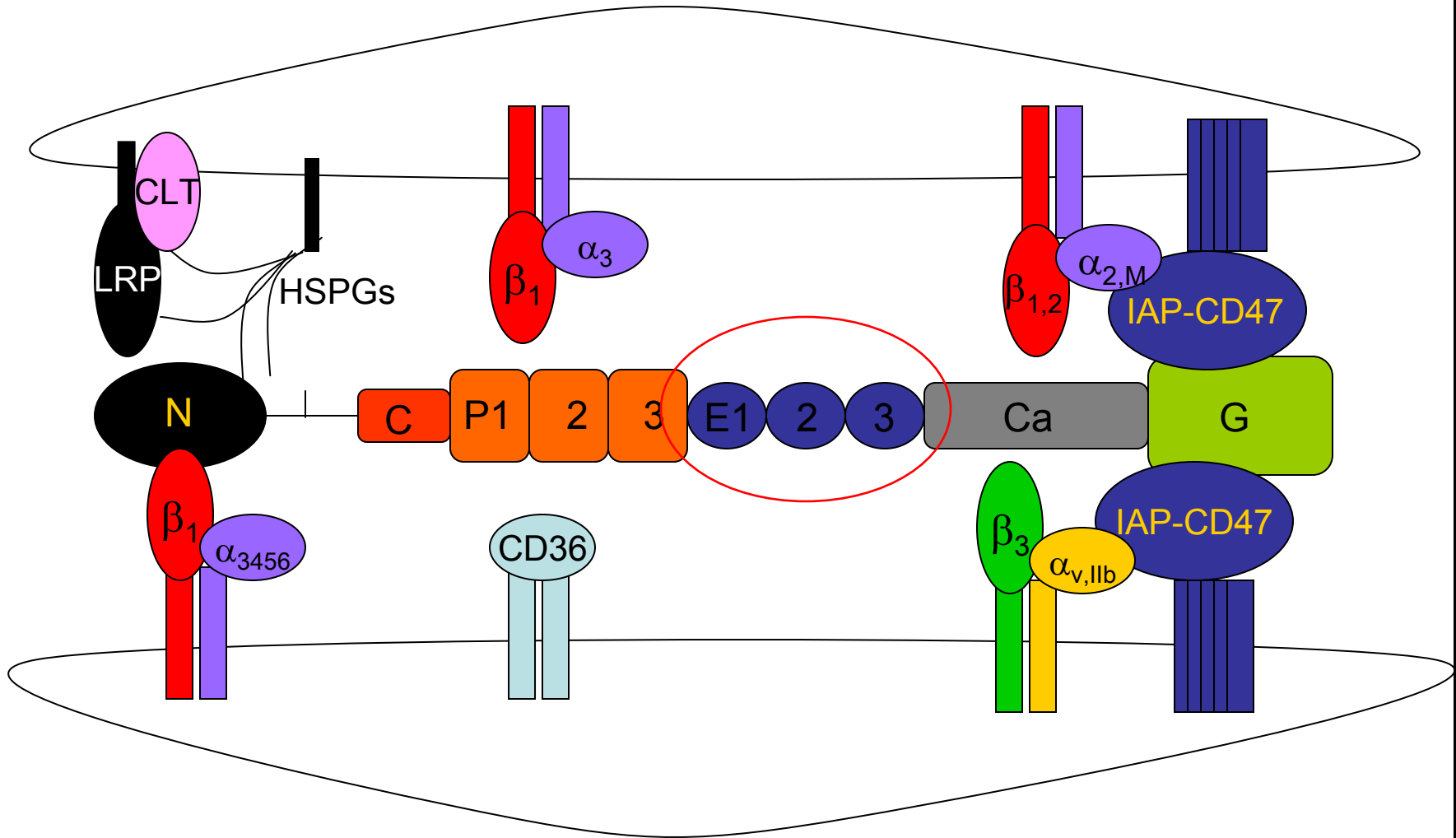
Which Domain of TSP is Synaptogenic?



Which Domain of TSP is Synaptogenic?



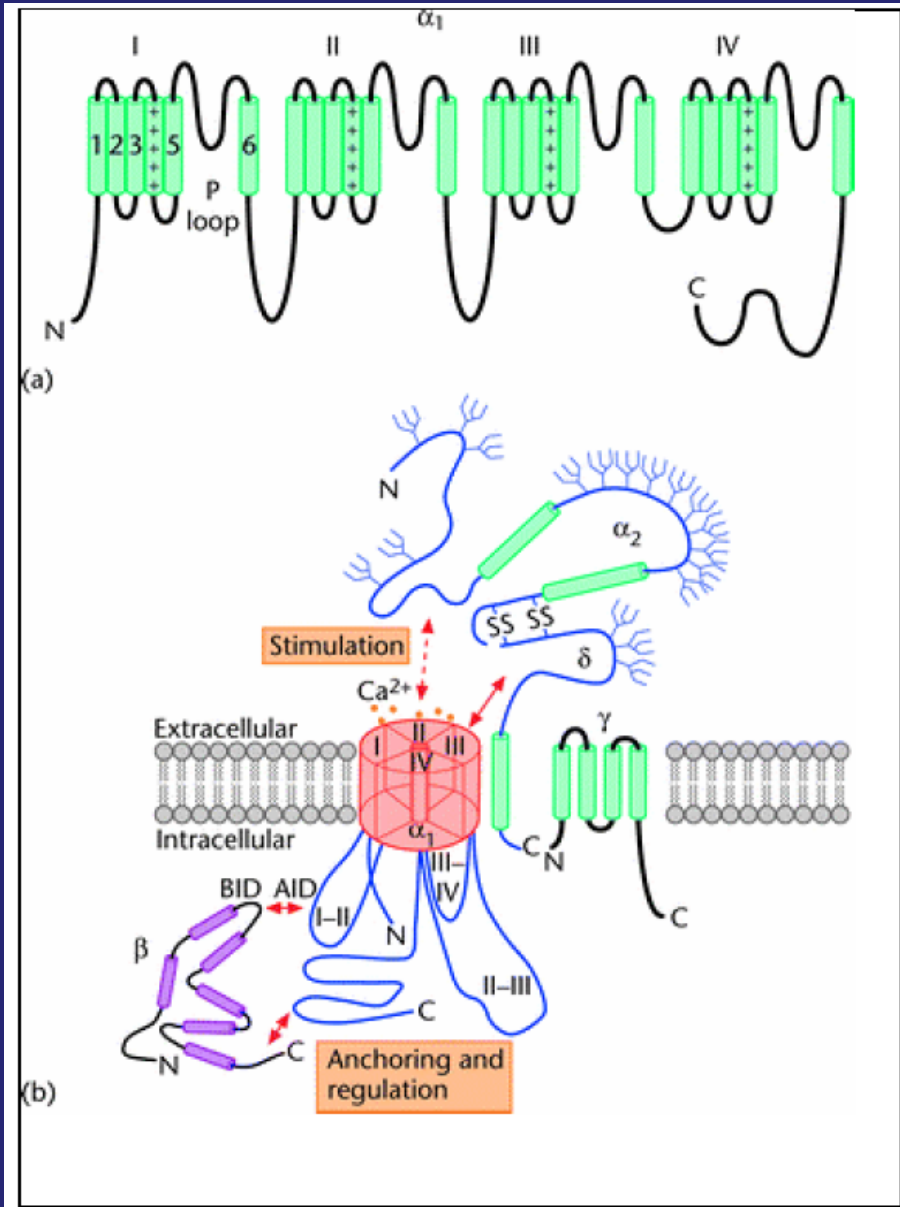
EGF-like Domains of TSP are Synaptogenic



Strategies to Identify the Neuronal Receptor for the EGF Domain

- Expression cloning
- Biochemical pull-downs
- Plow (2005) showed that TSP4 EGF domains interact with Itgam through its Von Willebrandt Factor A domain.
- Are there neuronal transmembrane proteins that contain the VWF-A domain ?

Calcium Channel Subunit $\alpha_2\delta$



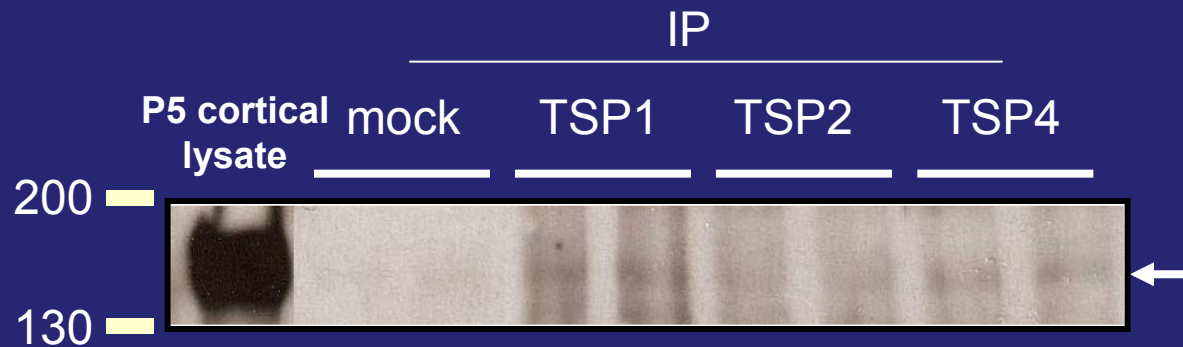
-Ubiquitously expressed by neurons including RGCs

- Pre and postsynaptic

-Post translational cleaved into α and δ subunits that stay attached by di-sulfide bridges

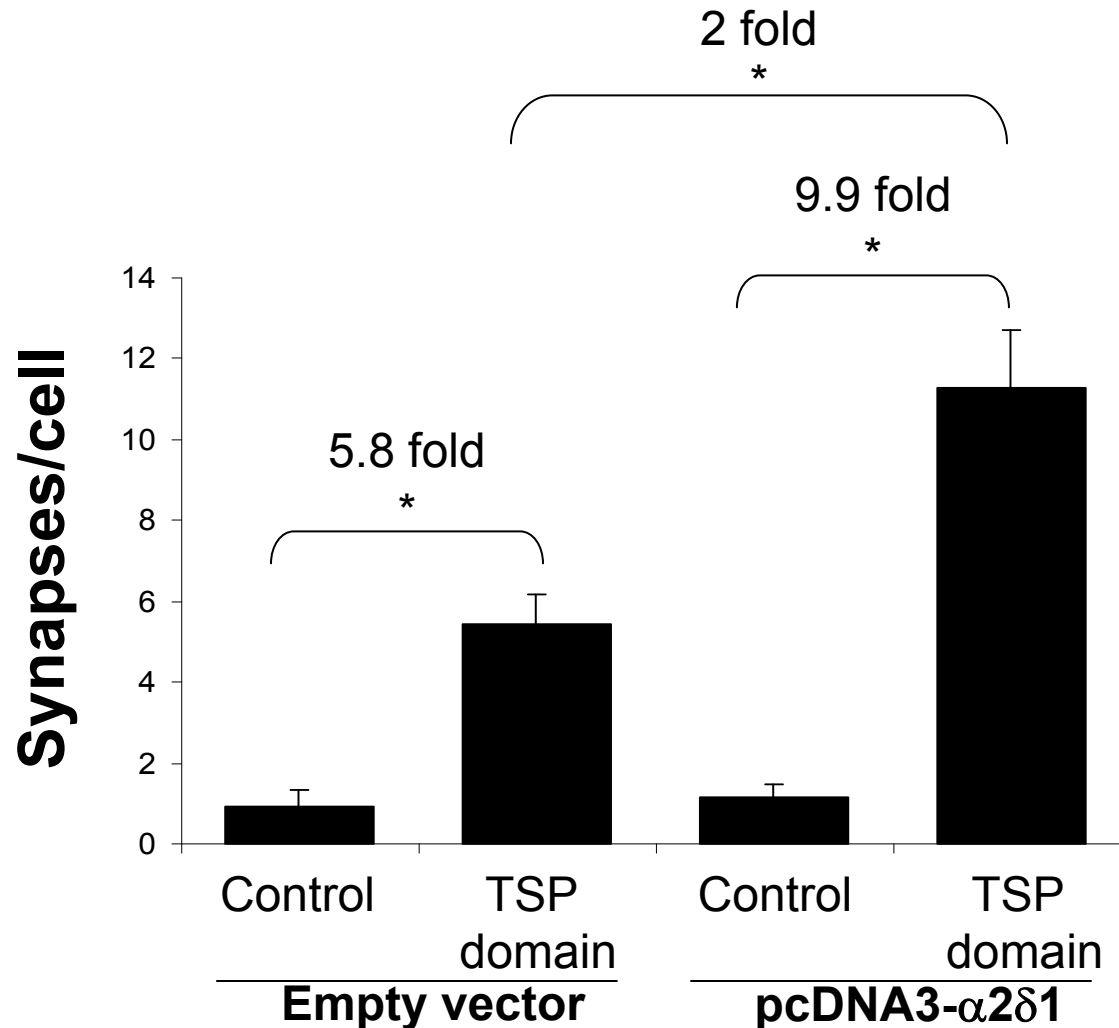
-Increases the number of calcium channels on the cell surface

Calcium Channel Subunit $\alpha 2\delta 1$ Immuno- Precipitates with Antibodies Against Thrombospondins

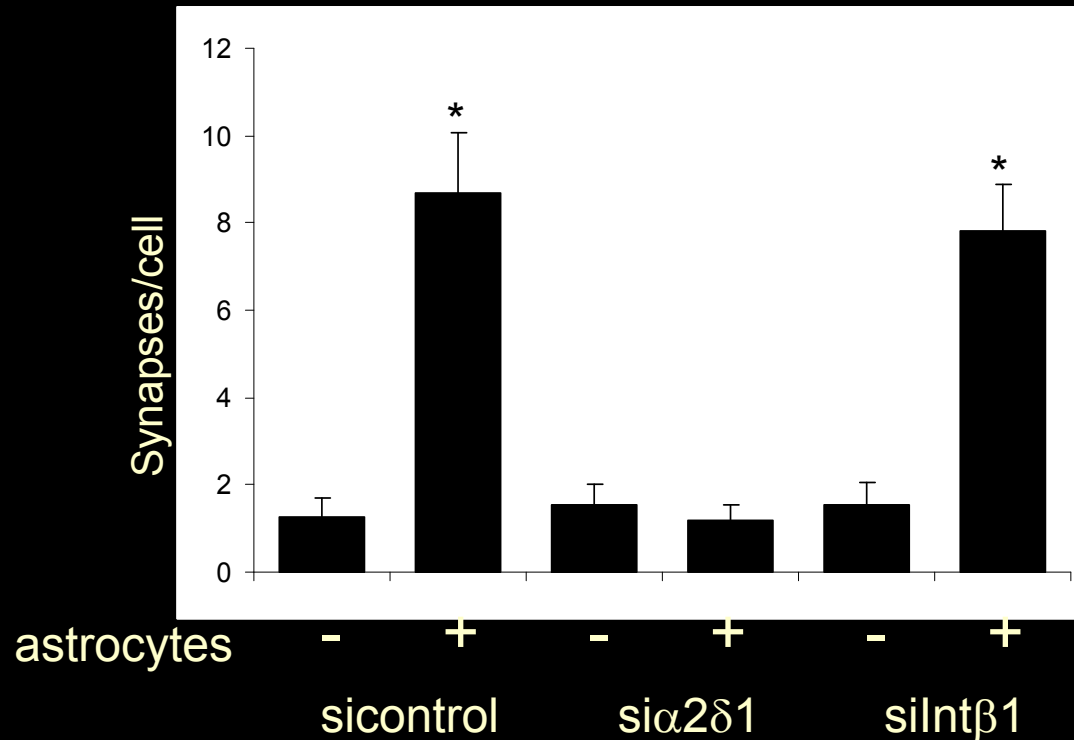


Is $\alpha 2\delta 1$ sufficient and necessary for TSP (and astrocyte) induced synapse formation?

Overexpression of $\alpha 2\delta 1$ in RGCs Enhances Thrombospondin Induced Synapse Formation in Vitro

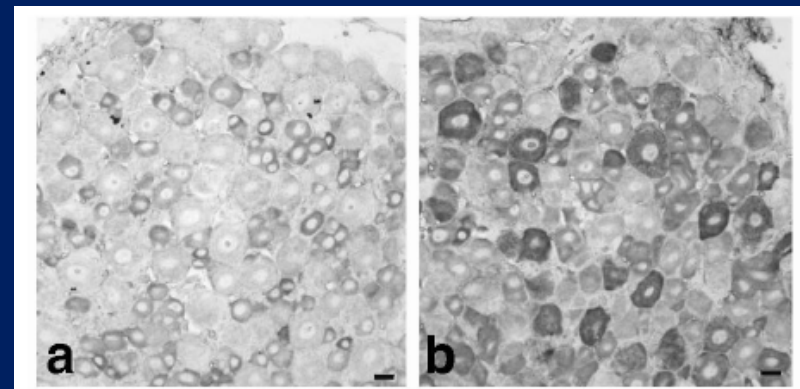
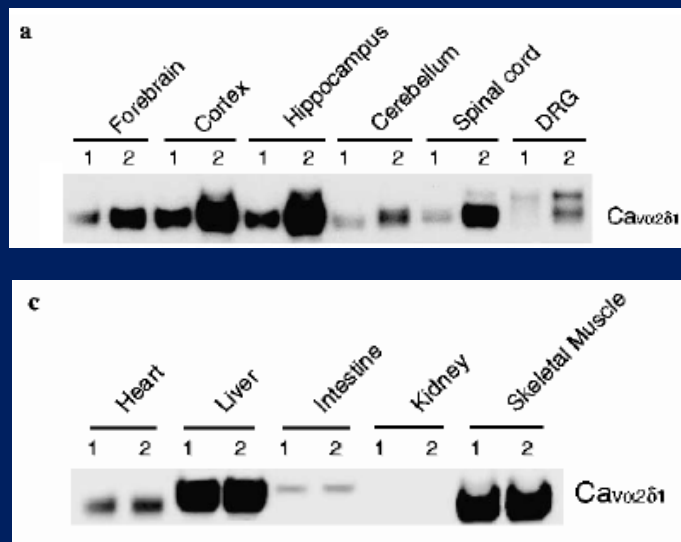


$\alpha 2\delta 1$ is Required for Astrocyte Induced Synapse Formation by RGCs in Vitro

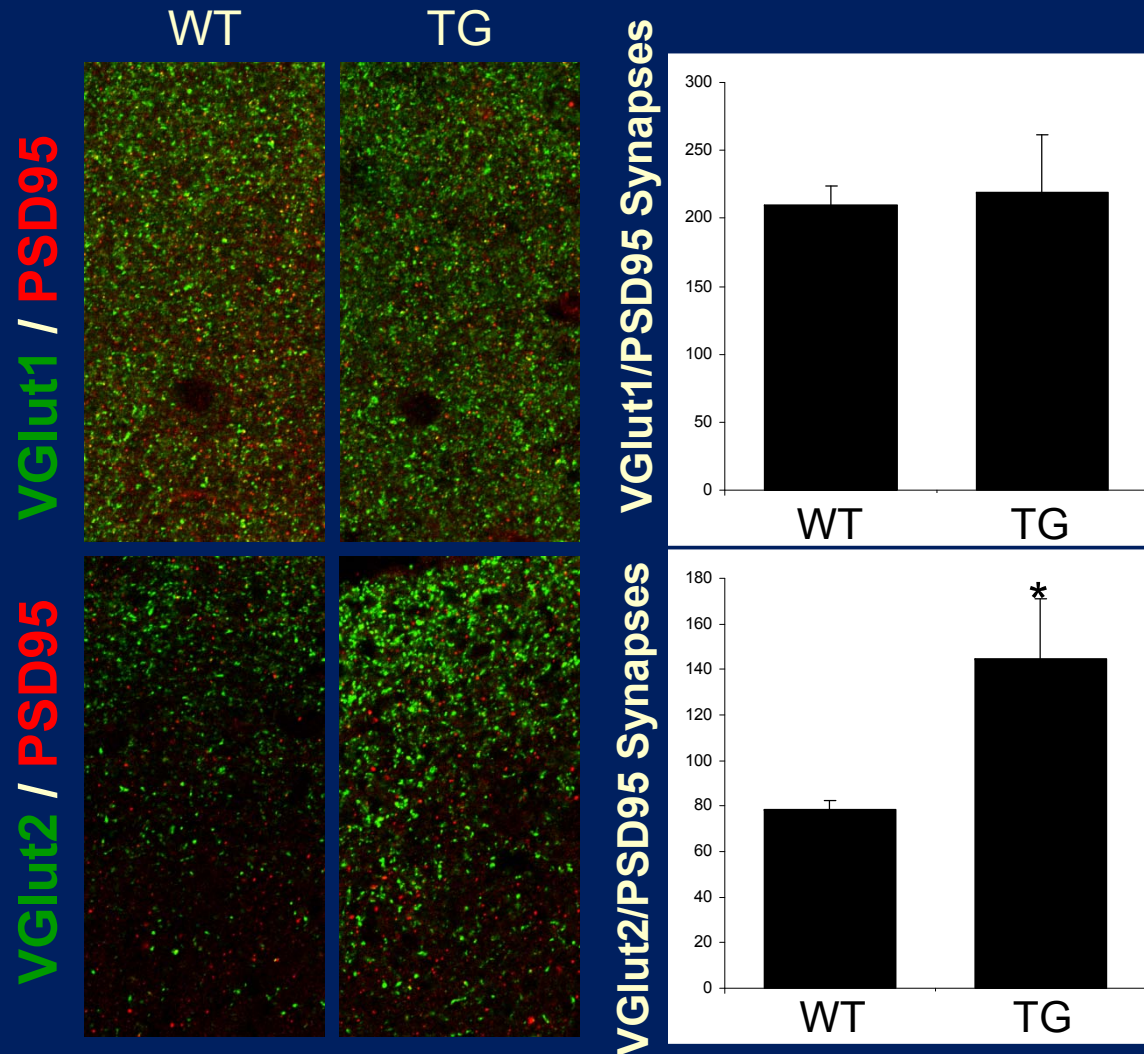


Does $\alpha 2\delta 1$ Promote Synapse Formation *In Vivo*?

- KOs are embryonic lethal
- TG mice overexpressing $\alpha 2\delta 1$ specifically in neurons was made by David Z. Luo and Guopeng Feng Labs to study the role of $\alpha 2\delta 1$ in neuropathic pain after spinal cord injury

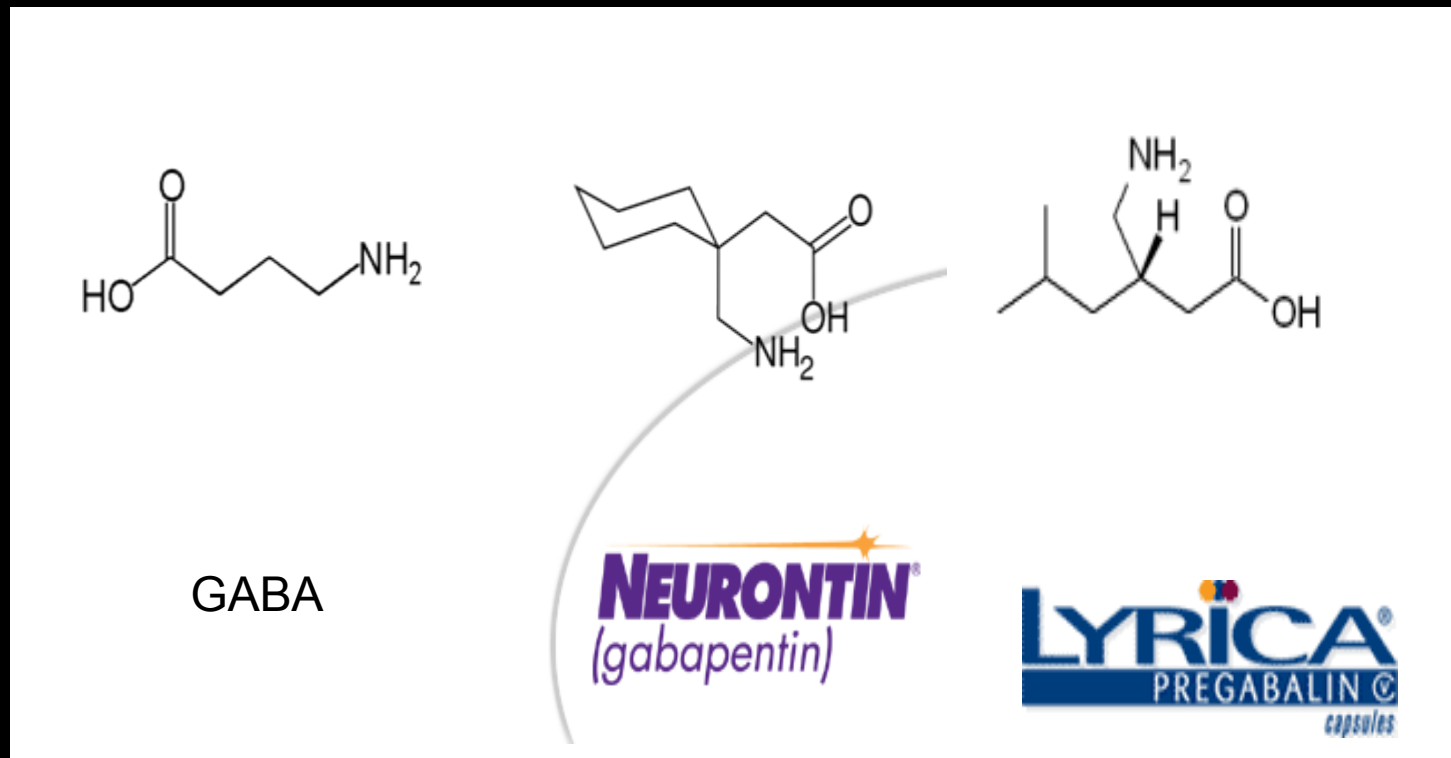


Overexpression of $\alpha 2\delta 1$ Subunit in Neurons Enhances Synapse Formation In Vivo



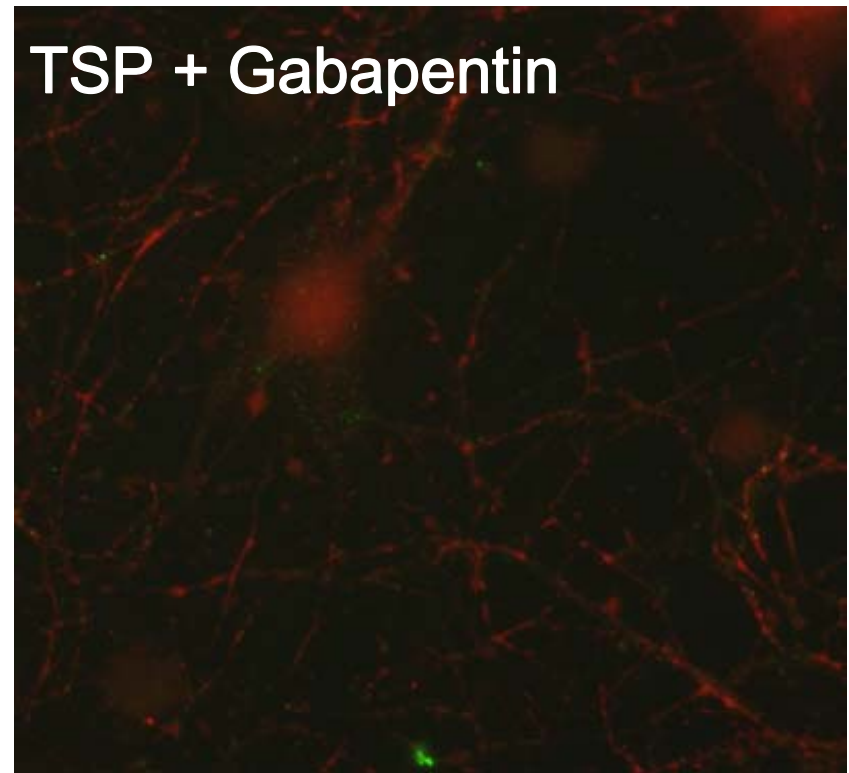
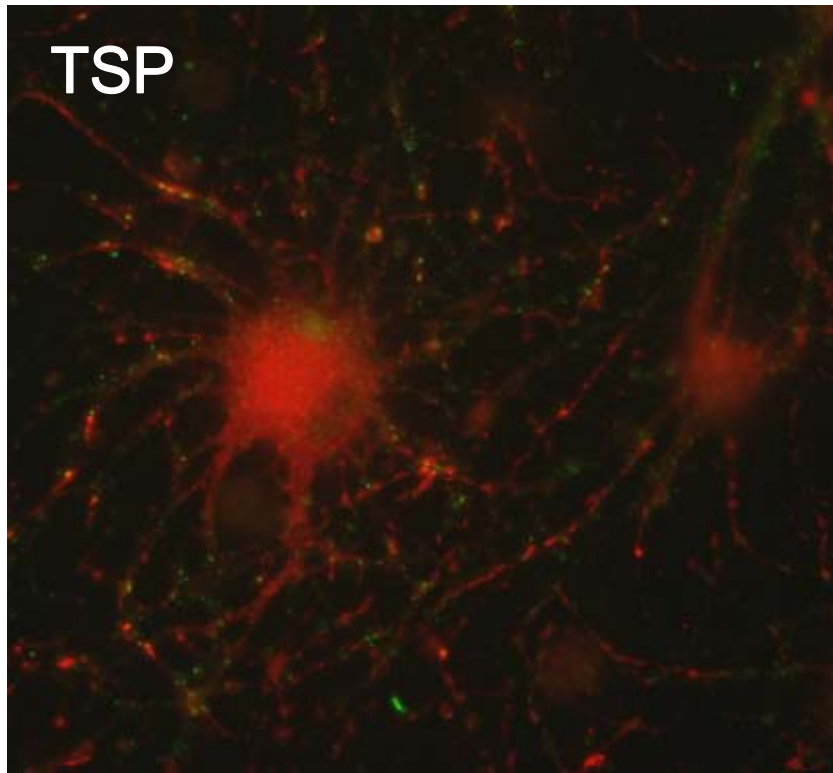
P21 cortices 4 TG versus littermate WT controls, * p<0.05

Calcium Channel Subunit $\alpha 2\delta 1$ is the Receptor for Gabapentin and Pregabalin

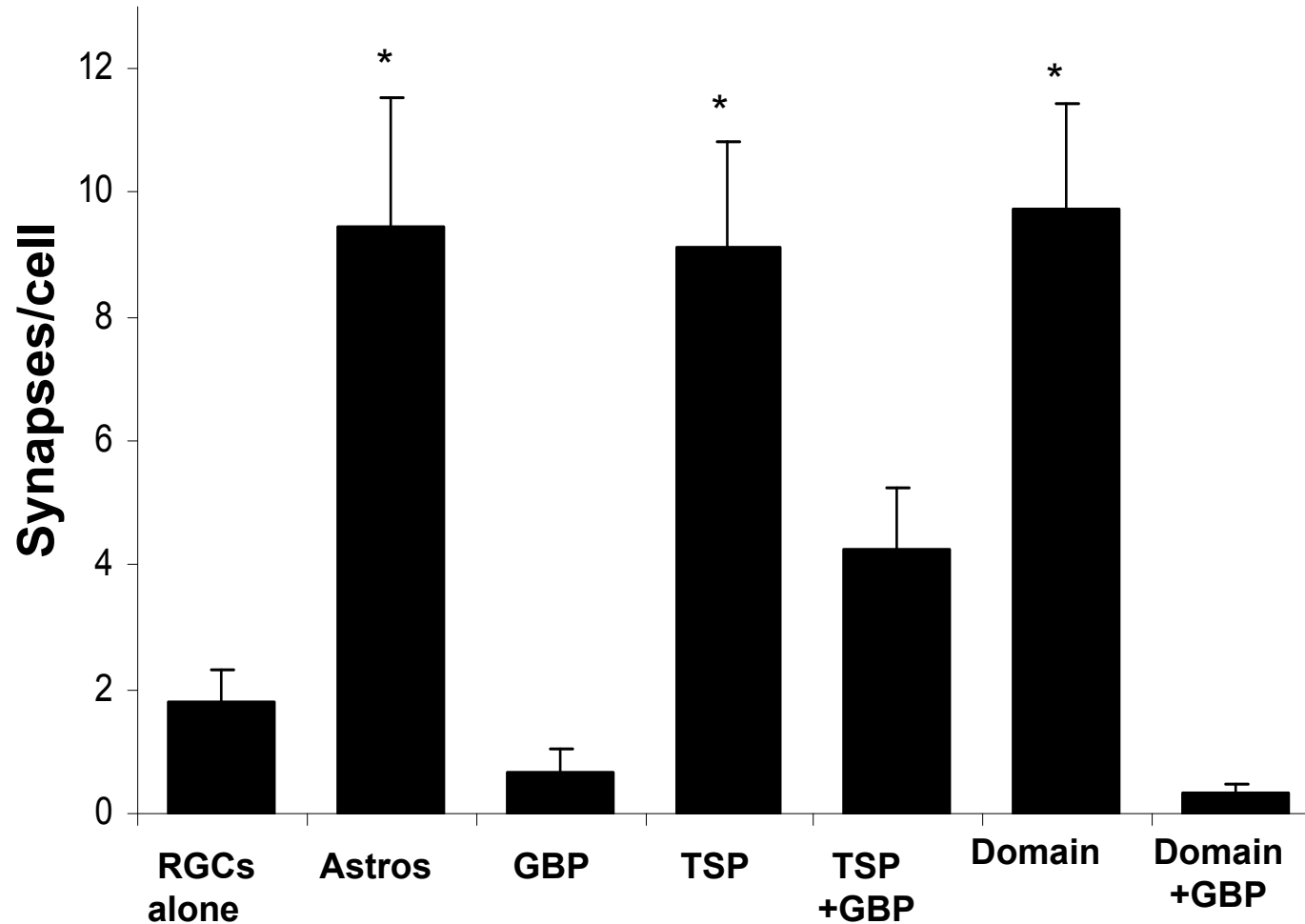


- Used to treat chronic pain and epilepsy
- Their mechanism of action is unknown

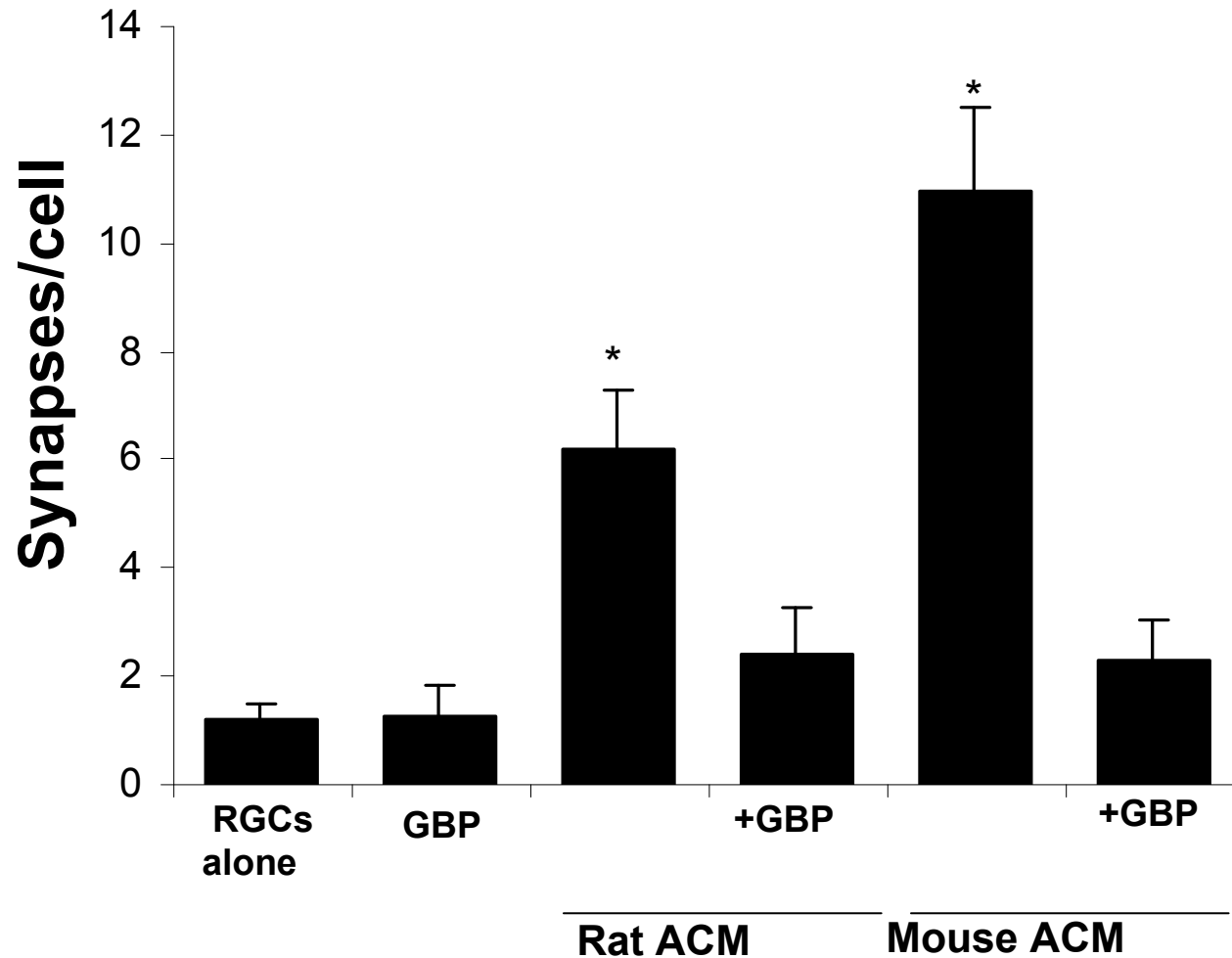
Gabapentin Blocks TSP-Induced Synaptogenesis



Gabapentin Blocks TSP-Induced Synapse Formation

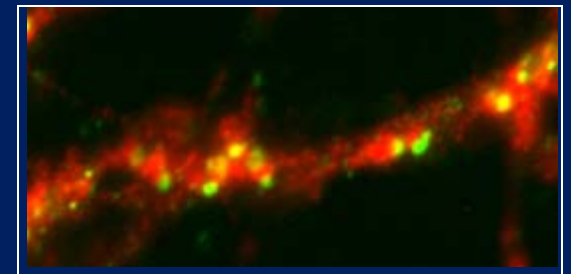
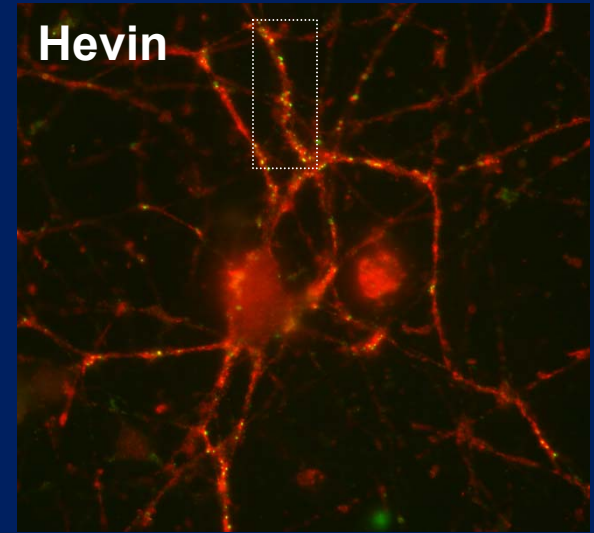
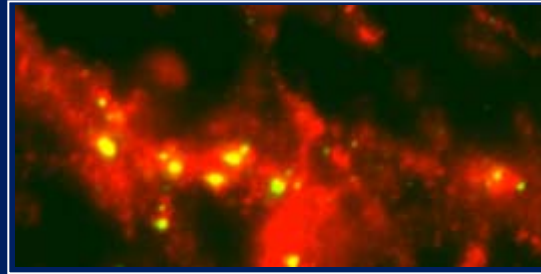
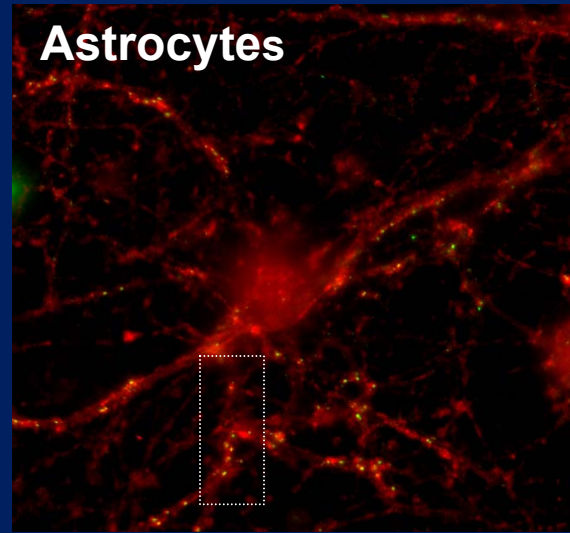
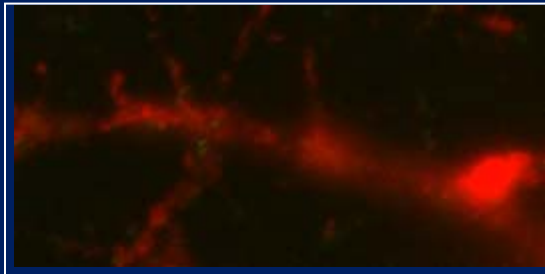
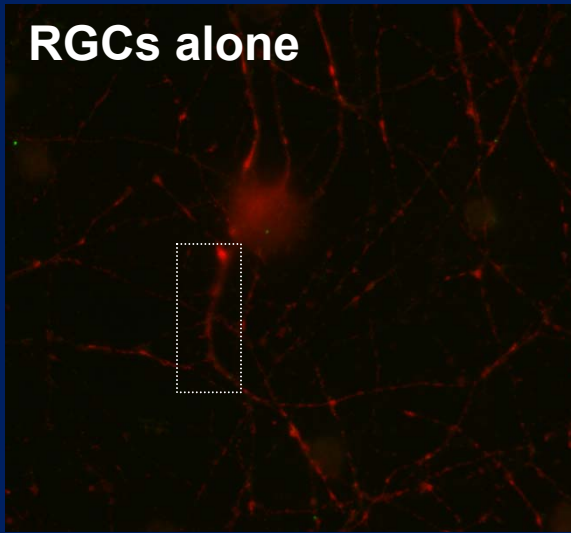


Gabapentin Blocks Astrocyte-Induced Synapse Formation



Does Gabapentin Block All Forms
of Synapse Formation?

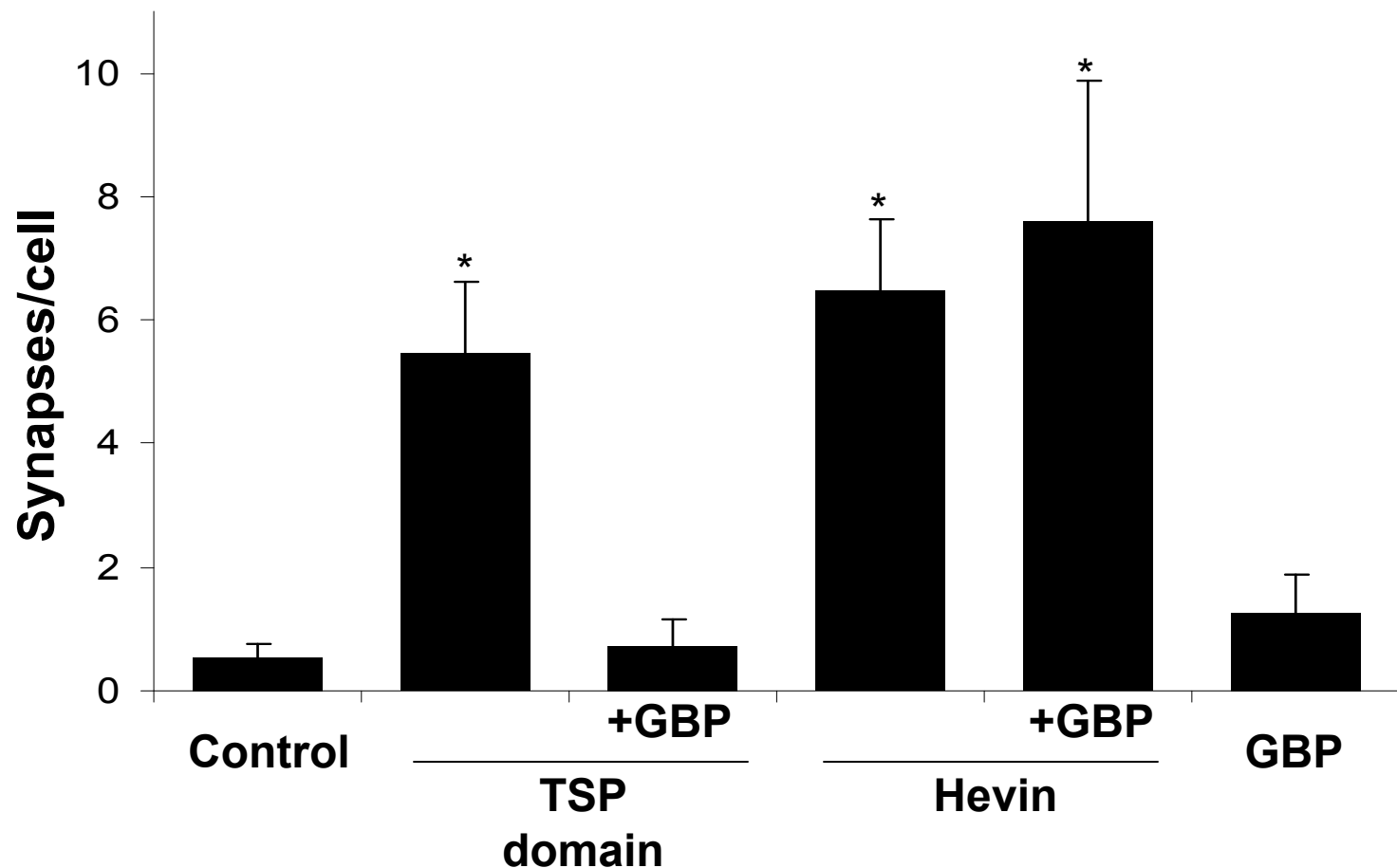
ECM Protein Hevin Induces Synapse Formation



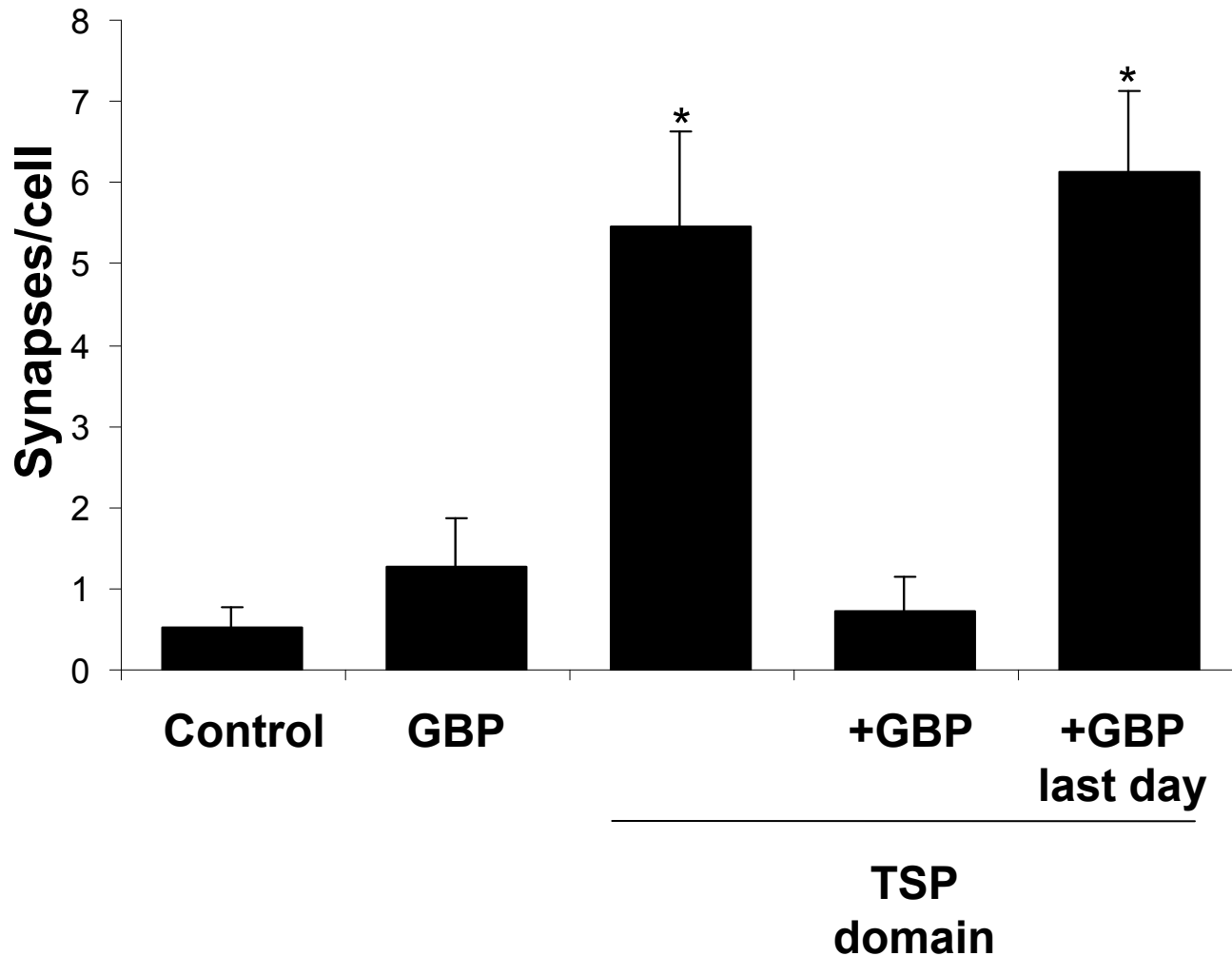
Synaptotagmin

PSD-95

Gabapentin Specifically Blocks Synapse Formation Induced by Thrombospondin

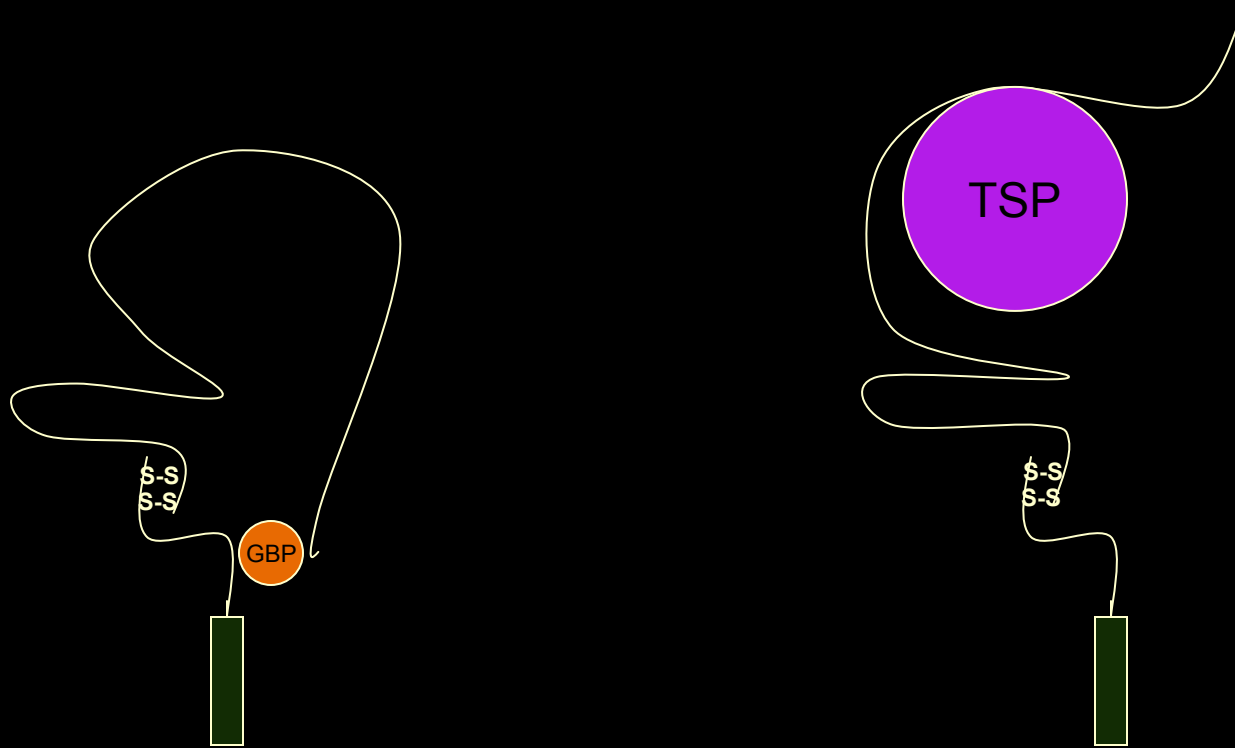


Can Gabapentin Dissolve Already Formed Synapses?

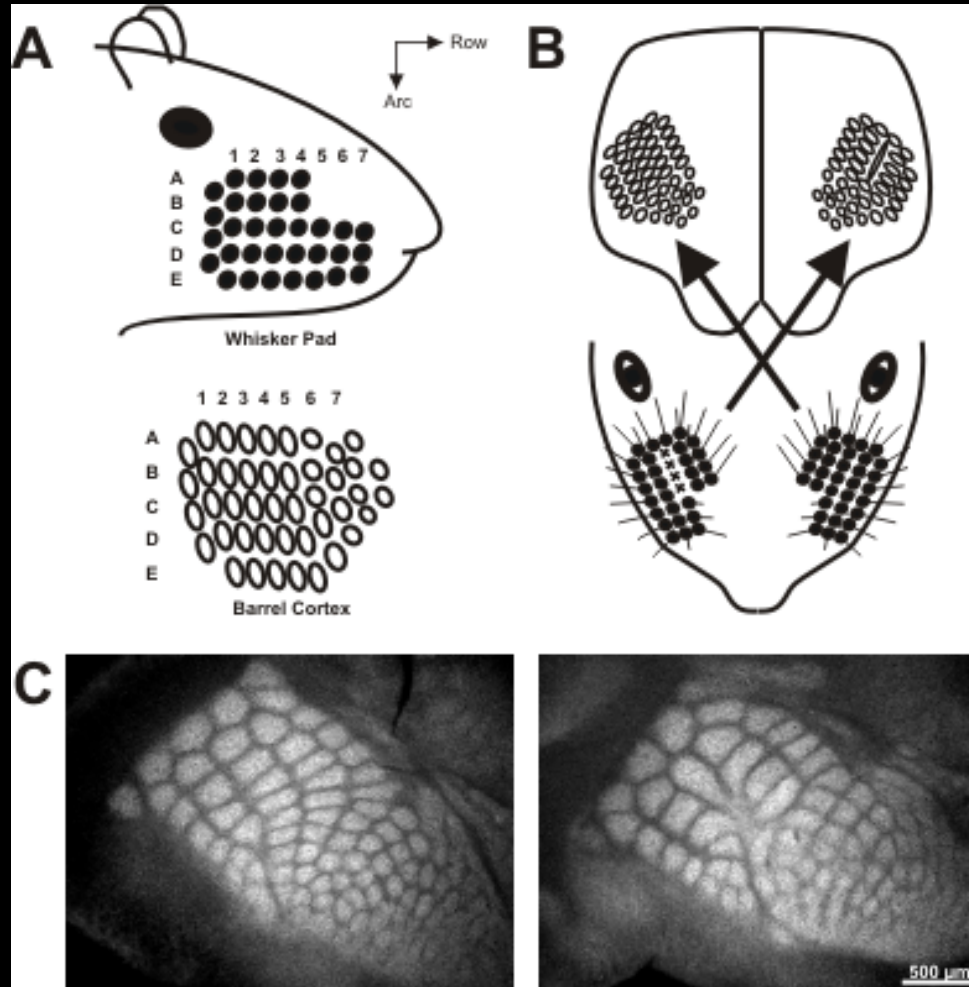


Gabapentin does not dissolve already formed synapses

How does Gabapentin Block TSP Induced Synapse Formation?



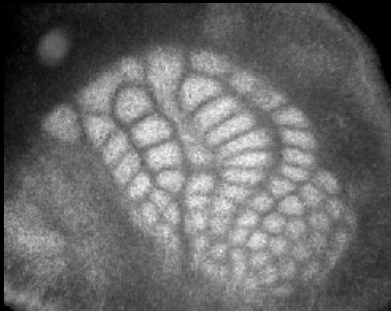
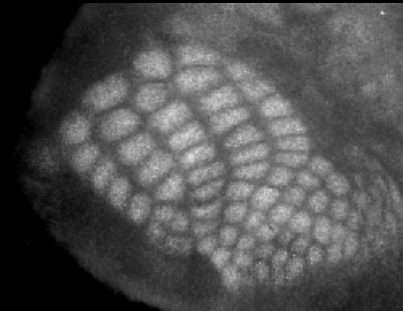
Does Astrocyte/TSP Induced Synaptogenesis Play a Role in Developmental Synaptic Plasticity?



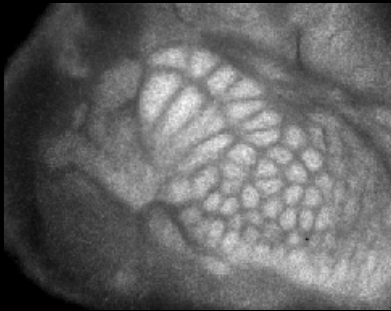
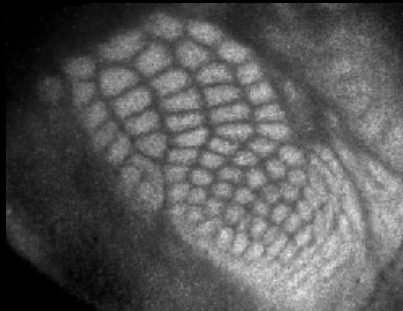
TSP- $\alpha 2\delta 1$ Interaction Plays a Role in Barrel Cortex Plasticity

Control side

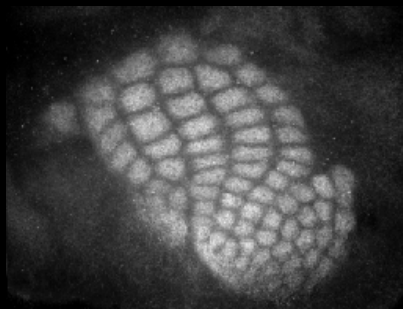
Lesion side



Saline Injected
No phenotype
0 out of 8



GBP Injected
Phenotype in 50%
4 out of 8



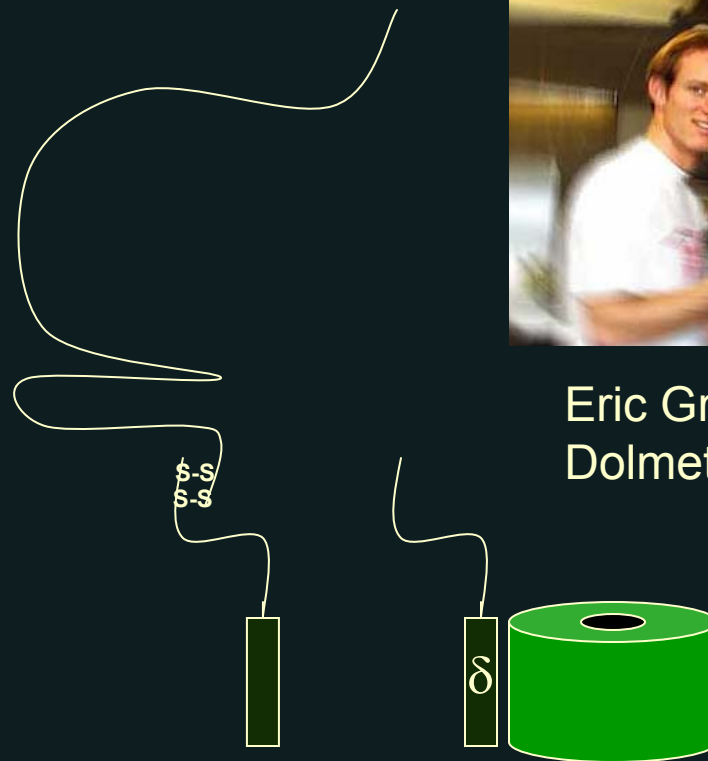
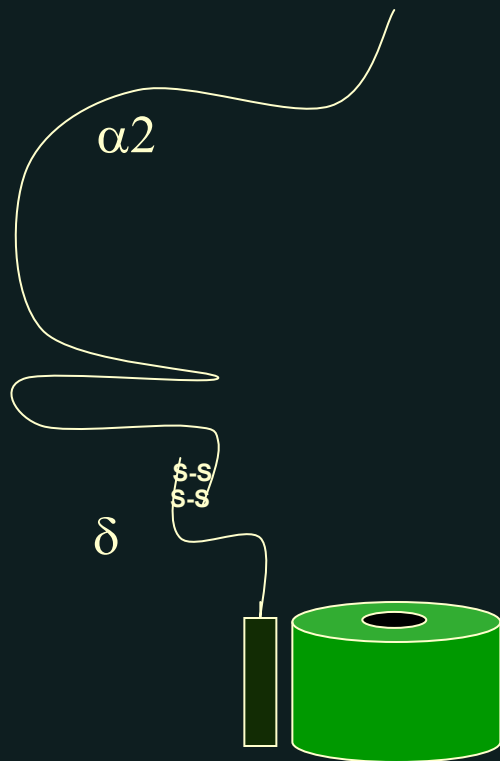
TSP1/2KO
Phenotype in 33%
4 out of 12

Summary

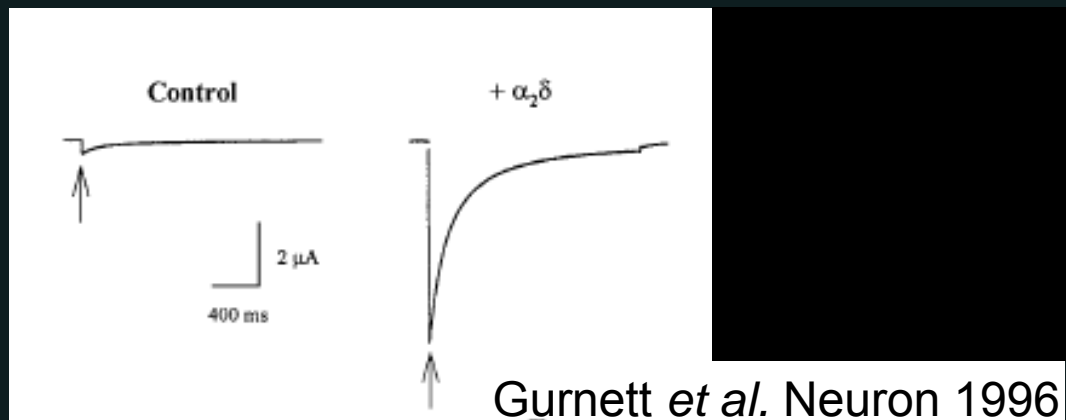
- TSPs induce synapses through their EGF-like domains
- The gabapentin receptor, calcium channel subunit $\alpha 2\delta 1$, is the synaptogenic TSP receptor
- Gabapentin blocks new synapse formation induced by astrocytes and TSP *in vitro* and *in vivo* (raising concern about use of drug in babies and in pregnancy)
- These findings add to the growing data that astrocytes promote synapse formation and plasticity *in vivo*
- Are calcium channels required for $\alpha 2\delta 1$ promotion of synaptogenesis?
- Is GABA a physiological ligand for $\alpha 2\delta 1$?

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Overexpression of δ Subunit Inhibits Enhancement of Calcium Currents by $\alpha_2\delta_1$



Eric Green
Dolmetsch Lab



Gurnett *et al.* Neuron 1996

Overexpression of δ Subunit Blocks TSP Induced Synapse Formation

